ELECTRON MICROGRAPHS OF INSECT TRACHEÆ[1]

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This paper presents electron micrographs of the tracheæ of mosquito larvæ, pupæ and adults (*Culex pipiens* L.) tracheæ, air-sacs and tracheoles of the adult worker honey bee (*Apis mellifica* L.) and tracheæ of the adult American cockroach (*Periplaneta americana*). Many of the minute details are considerably beyond the resolving powers of the light microscope and so are shown for the first time.

A discussion of the application of electron optics to insect cuticle studies is given in another paper (Richards & Anderson, 1942.) Descriptions and discussions of electron microscopes are given by Zworykin (1941); Zworykin, Hillier & Vance (1941); Hillier & Vance (1941); Smith (1941); Burton & Kohl (1942); Anderson (1942), etc.

TECHNIQUE

The technique is relatively simple. Tracheæ and air-sacs were dissected out and passed through several changes of distilled water for 1–24 hours to remove all the surrounding cells. The remaining clean intima is placed on thin collodion membranes across a fine-mesh wire screen (Richards & Anderson, 1942). Since best resolution is obtained from single layers, most of the tracheæ were deliberately torn open.

Very small tracheæ and tracheoles cannot be readily handled as such. Whole organs (nerve cord, portions of alimentary tract, etc.) were removed, the cells dissolved away by immersion in several changes of distilled water (5½ to 24 hours), and the re-

[1] Thanks are due to the Radio Corporation of America and to Dr. V. K. Zworykin for use of the electron microscopes in the RCA Research Laboratories, and to the National Research Council's Committee on Biological Applications of the Electron Microscope, through which arrangements for this work were made.

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maining tissue teased apart and mounted on a collodion film across a wire screen.

Numerous specimens were also mounted in Apathy's gum syrup[4] for check study with the light microscope. Similar permanent mounts were eventually made of specimens studied in the electron microscope. Serial sections were also used in these comparative studies.

LITERATURE ON TRACHEAL STRUCTURE

Most of the papers on insect tracheæ deal either with their distribution or functioning; literature on tracheal structure is not extensive. It is generally stated that there are tracheæ, tracheoles and air-sacs. The tracheæ are cylinders (sometimes collapsible) supported by many helical thickenings called tænidia; the tracheoles are minute (less than 1 μ diameter) terminal branches of the tracheæ usually distinguished by the reported absence of tænidia; the air-sacs are distensible sacs, without tænidia, on the tracheæ of some insects. In the large tracheæ of some species of insects the walls are thick, and staining reactions show they possess the same layers as the cuticle of the exoskeleton (Weber, 1933); in other species the tracheal walls are extremely thin and no such differentiation is demonstrable.

Variations have been studied principally by Marcu (1929–1931) who found large spines on the tænidia of certain beetle tracheæ, linear thickenings in the tracheæ of other beetles and an irregular meshwork in the membrane between tænidia of various bees and wasps. See also Dujardin (1849), Stokes (1893), Packard (1898), Weber (1933) and Wigglesworth (1939).

TRACHEÆ OF THE MOSQUITO (CULEX PIPIENS)

LARVA: The intima of the two main longitudinal tracheal trunks is a thin, brown membrane with typical helical thickenings (tænidia). The thickness of the membrane was not determined accurately but cannot be more than 0.02 μ in dried tracheæ. The tænidia are readily visible with the light microscope since they are 0.3 to 0.7 μ wide (in areas shown in the micrographs the range

[4] 50 grams gum arabic, 50 grams sucrose, 50 cc. distilled water and 1 cc. formol. Dissolve the gum arabic in warm water, add sucrose and dissolve, cool, filter and add formol.

is 0.3–0.6 μ); they are opaque to 50 kV and 100 kV electrons (Fig. 3) but are readily penetrated by 200 kV electrons (Fig. 1); obviously a single tænidium may end either by anastamosis with another (Fig. 1) or tapering off into the membrane (Fig. 4). The tænidia and in some regions the membrane also bear minute spines (microtrichiæ) which are not limited to the region adjacent to the spiracles but occur throughout the length of the longitudinal trunks (not on the lateral branches, Figs. 1, 3, 4). These spines are of quite diverse sizes and shapes (Fig. 3); the average length is about 0.1 μ with a normal range of 0.08–0.15 μ, but much smaller ones also occur with the extremes being mere lumps not over 0.02 μ.[5] Most of the spines do not project into the lumen of the trachea but arise from the sides of the tænidia and project parallel to the membrane; about 25 per cent of them project more or less into the lumen but very few (less than 10 per cent) project directly into the lumen (Fig. 1).

Figure 3 has resolution of points to 0.004 μ or slightly smaller. The membrane is uniform. Accordingly this membrane in the dried state cannot have any pores larger than 0.004 μ diameter.

The large lateral tracheæ arising from the main trunk have a similar appearance under the light microscope but electron micrographs show that they lack the minute spines on the tænidia and membrane (Figs. 5–7). The thickness of the membrane as determined by the thickness of the dark rim along tracheæ that have not collapsed is 0.01 μ to less than 0.02 μ (Fig. 5 is especially favorable for this measurement because it is taken at a higher voltage than the other similar figures). Similar but less accurate figures may be obtained by comparing the density of the tracheal membrane with that of the collodion membrane; this is readily done at points where the tracheal membrane overlies a hole in the collodion film (arrow in Fig. 7).[6] How much shrinkage is involved in drying is not known. The tænidia shrink less than 25 per cent (likely much less); even if we allowed such a high value

[5] These clearly not due to foreshortening as can be shown by comparison with figure 1.

[6] Allowance should be made for differences in density. The specific gravity of the tracheal intima is not known but the specific gravity of pure chitin is 1.40 (Sollas, 1907) whereas the specific gravity of collodion (nitrocellulose) is approximately 1.66 (Hodgman, 1935).

for shrinkage of the intertænidial membrane on drying this membrane would still average less than 0.02 μ thick.

In most of the electron micrographs the tænidia appear as though outside the intervening membrane (Fig. 5). This must be due to irregular shrinkage since some of the micrographs show the tænidia in their normal position on the lumen side of the trachea (Fig. 6).

Most striking in appearance are the bands of large spines that encircle the main tracheal trunk and interrupt the tænidia (Fig. 4). These seem not to have been reported heretofore although they are readily visible with the light microscope. They occur as bilaterally symmetrical segmental bands immediately posterior (4–15 tænidia) to the origin of the main lateral segmental tracheæ. There are therefore eight pairs of these in the abdomen, all far removed both from the larval spiracles and from the sites of the future adult spiracles. These bands consist of spines which project directly into the lumen of the trachea. In the electron micrograph the spines appear shorter than they actually are (due to foreshortening); measurements in sections using the light microscope show these spines are 2–3 μ, mostly around 3 μ long. Between the spines is a meshwork of thickenings of the intima. This meshwork connects with the adjacent tænidia and runs into the spines in such manner that it seems the spines are formed from the outward projection of a focal cone of the meshwork.

Study of serial sections with the light microscope shows no obvious anatomical association of these spinose bands except with the trachea itself; also no differences between the cells which underlie this and other parts of the trachea. Examination of shed skins shows that in part at least these bands serve as fracture points for the main tracheal trunks during moulting,[7] but the tracheal trunks may also break at other points. Their elaborate structure suggests that they might also serve some other purpose. These tracheal trunks pulsate rhythmically (Babak, 1912). When the pulsating trachea is almost completely collapsed in the regions of these bands, the spines interlock and partially occlude the trachea. Conceivably these intermeshed spines could serve as a sieve to strain dust particles from the air but this would not

[7] Suggested by Dr. John B. Schmitt.

seem of great importance since they are far removed from the spiracles and since in the many specimens examined no accumulation of debris was ever seen in the trachea. Another suggestion is that these bands when interlocked serve as check valves to assist in the movement of air along the pulsating trachea.

Shed skins of mosquito larvæ gave similar micrographs but also revealed one additional point. In general, the tracheal intima is shed and pulled out during each moult but it would seem that this does not always occur. Figures 16–17 are stereoptic pictures of large lateral tracheal branches from the shed skin of a third instar larva. Examination of these pictures in a stereoscope shows that the smaller helix is within the larger helix. Obviously, then, these are double tubes (such double tubes were observed commonly on the fluorescent screen). Conceivably the tracheal intima might split but the inner tube is definitely smaller; it seems more likely that the inner tube represents the tracheal intima of the second instar larva, that this intima was not pulled out at the second moult, that the third instar larva had a double intima, and that both of these were withdrawn together at the third moult. If this interpretation is correct, it is interesting to note the close agreement of the tænidia in successive instars.

PUPA: The main longitudinal tracheal trunks of the abdomen lack the dark pigment that is present in larval tracheæ. They show spinose bands and minute spines on the tænidia similar to those of the larvæ. Some of the tracheæ are indistinguishable from larval tracheæ, but some of them are strikingly different in that the intertænidial membrane instead of being uniform has a definite reticulation of thickenings (Fig. 8).

ADULT: Only a few tracheæ from near the sixth abdominal spiracle were examined. The intertænidial membrane is not perfectly uniform, and the tænidia, instead of always being helices are some helices, some rings (Fig. 9).[8]

TRACHEÆ, AIR-SACS AND TRACHEOLES OF THE HONEY BEE (APIS MELLIFICA)

In the tracheæ of adult worker honey bees Marcu (1930) has already described and figured (photomicrographs) what he con-

[8] That the tænidia may sometimes be rings rather than helices is already known. See Snodgrass 1935, p. 448.

sidered braces extending from the tænidia onto the intertænidial membrane. Electron micrographs show the details are much finer than this. The tænidia have wavy edges, and an irregular meshwork of thickenings is present in the intertænidial membrane. The pattern is not constant but two principal types were observed: one in which the thickenings are more or less bead-like or knobbed (Fig. 10) and one in which there are few beads or knobs (Fig. 11). The larger of these thickenings approach the size of the tænidia; the smallest of the clearly resolved thickenings approximate 0.015 μ broad. Tracheoles did not possess such thickenings but we did not check a full graded series to determine at what size tube they disappear.

The thickness of the tracheal membrane cannot be determined accurately from the thickness of the edge of the tube because of interference by the thickenings. It seems to be of the general order of 0.01 μ in dry tracheæ.

The air-sacs have a very irregular meshwork of thickenings of various sizes (Fig. 12).

Considerable time was spent searching for tracheoles. Many were seen on the fluorescent screen of the microscope but only three less than 0.5 μ in diameter were sufficiently free of debris for micrographing. All sizes of tracheæ from the large main tracheæ to small tracheæ less than 0.2 μ in diameter after drying[9] were examined. All of these possessed tænidia (Fig. 15). Accordingly the main morphological characteristic for differentiation between tracheæ and tracheoles is invalidated. The tænidium (about 0.025 μ wide[10]) in these tracheoles, like that in large tracheæ, is not a continuous helix but it differs from the tænidia of large tracheæ in that one thread makes 6–8 turns around the tube and then ends, overlapping only slightly or not at all with the next helix. Other micrographs not being published show a

[9] The two smallest seen clearly were each collapsed as shown by the shape of the helical thickening. They each had a calculated diameter of 0.175 μ. Allowing for 10 per cent shrinkage (Richards & Anderson, 1942) and the probable error in calibration and measurement, these two are still less than 0.20 μ in diameter after drying.

[10] Equally small and even smaller anatomical details are found in the brilliant iridescent wing-scales of the butterfly *Morpho cypris* (Anderson & Richards, 1942).

tracheole of approximately 0.2 μ diameter ending blindly with the tænidium extending all the way to the closed end. Another micrograph of a tracheole about 0.35 μ in diameter shows several denser regions where the tænidia are more tightly coiled.

The intertænidial membrane of tracheoles is very thin. The specimen shown in figure 15 obviously moved during micrographing. This is shown by the double silhouette line indicated by the arrow. Using the clearer outer line for measurement, this membrane would be only 0.005 μ thick in dry state. The probable error here is high because such a magnitude is near the limit of resolution, but certainly this membrane must be considerably less than 0.01 μ thick.

TRACHEÆ OF THE COCKROACH (PERIPLANETA AMERICANA)

Tracheæ were taken from branches around the gut. The larger tracheæ differ from both of the preceding species in having circular or oval thickenings in the intertænidial membrane (Fig. 13). These are 0.15–0.25 μ broad and of approximately the same thickness (shown by one micrograph in which the membrane is torn and curled over, showing the thickenings as lumps along the edge). Small tracheæ (1–2 μ diameter) have only few or none of these thickenings.

The thickness of the intertænidial membrane was not determined accurately. It seems to be slightly thicker than in the preceding two species but is not over 0.05 μ thick.

One small trachea about 1.5 μ in diameter showed the tænidia as short helices, incomplete rings and complete rings (Fig. 14).

DISCUSSION

Aside from the unsuspected minuteness of details shown by these micrographs of tracheæ, four points warrant brief discussion. These are: (1) what is a tracheole? (2) what is the origin and development of tænidia and other thickenings? (3) what is the reason for the uniformity of the very thin membranes? and (4) what are the smallest sizes of cuticular structures that can be seen with the light microscope?

(1) The common definition of a tracheole is a small tube (less than 1 μ) lacking a tænidium. This is obviously invalidated by

the present work. Other characteristics that have been given are that they arise intracellularly (Tiegs, 1922; Wigglesworth, 1939), that they anastamose or end blindly (Wigglesworth, 1939), that they are freely permeable (Wigglesworth, 1938; Bult, 1939), that they are completely dissolved at the time of moulting whereas the tracheal intima is shed (Tiegs, 1922), and that they are not chitinous (Campbell, 1929). While the above may be perfectly good differences they are not always useful in differentiating between tracheæ and tracheoles. Probably the best characteristic left is the intracellular origin of tracheoles versus the origin of tracheæ from a tube of cells. But it is not certain that this criterion is always valid though it would seem necessary for tubes less than 1 μ in diameter to develop in this manner, and it is not possible to apply this differential characteristic in those cases where the tracheole passes gradually into the trachea (*i.e.*, no tracheal end cell present).

(2) The tænidia have been said to originate as folds in the tracheal intima. This view was put forward by Macloskie (1884) who considered the tænidia as hollow tubules. Obviously the tænidia are solid structures in the two species treated in this paper, and they are generally treated as solid in current textbooks although some recent authors refer to them as originating from folds (Wigglesworth, 1939). One can imagine the larger elements of the thickenings in the bee air-sac (Fig. 12) arising in this manner. It is conceivable that the tænidia might originate as folds but their density shows that they are not tubes and that the folds would have to act as regions for the accumulation of further secreted material. An alternative view holds that the tænidia represent a discontinuous exocuticula (Weber, 1933). The similarity in appearance of tænidia from various sources suggests a common explanation. A helical thickening only 0.025 μ broad suggests that large-scale polymer phenomena may be involved as at least the basic mechanism in tænidial development. This polymerization might well have similarities to the polymerization involved in the differentiation of the exocuticle of the exoskeleton.

Diversity of pattern of the membrane thickenings and smooth membrane in mosquito larvæ complicate interpretation both of

these thickenings and of tænidia. The smaller meshwork of the tracheæ of bees and mosquito pupæ (Figs. 8, 10, 11) certainly does not look like a pattern of folds, and the lumps in cockroach tracheæ seem even less like folds (Fig. 13), but these might represent polymerization patterns with differences between various tracheæ correlated with differences in chemical structure (Campbell, 1929; Wigglesworth, 1939). Certainly the magnitudes are such as to suggest a chemical rather than a morphological explanation.

(3) There is general agreement that the larger tracheæ contain chitin (Koch, 1932; Wigglesworth, 1939). In some cases (*e.g.*, mosquito larvæ) they certainly contain pigment. Probably like the cuticle of the exoskeleton they also contain protein. The intima of large tracheæ seems to be composed of two layers as is the general exoskeleton: a hydrophobic epicuticle and an underlying chitinous endocuticle (or equivalents). In view of the uncertainty concerning the chemical constitution of tracheæ it is not possible to interpret the fact that tracheæ show uniform membranes which when dry are only 0.01–0.02 μ thick.

The membranes of tracheoles are thinner than those of tracheæ. This might be due to the probable absence of a hydrophobic lining or to a different chemical constitution (no one has been able to demonstrate the presence of chitin in the walls of these minute tubules. See Wigglesworth, 1939).

Yet these membranes are so perfectly uniform both over considerable areas in one trachea and from one trachea to another that their origin could be most readily visualized as a mono-layer. But apparently they cannot represent a mono-layer of chitin crystallites.[11] It remains to be seen whether a polymer or lattice unit will be found of sufficient size to account for the thickness of these thin membranes.[12] Obviously these remarks do not pertain to thick-walled tracheæ such as figured by Weber (1933, Fig. 393).

(4) Using the light microscope there is no difficulty in discerning the tracheal membranes in cross sections of tracheæ despite

[11] For size of chitin micellæ see Clark & Smith (1936).

[12] The fact that the smaller thickenings in the honey bee tracheæ are approximately twice the thickness of the membrane may be of some significance.

these membranes being only 0.01–0.02 μ thick. Usually the membrane is accentuated by the surrounding cells but it may be seen even when alone. The much larger thickenings in this membrane and the barbs on the tænidia of mosquito larvæ are scarcely discernible though by careful focusing slight irregularities can be detected (Fig. 2). Increased distinctness can be obtained by mounting air-filled tracheæ in Apathy's gum syrup and selecting points where the air-bubbles accentuate the contrast. In a few such mounts of mosquito tracheæ the barbs (0.1 μ) on the tænidia are fairly clear though they all look alike, and the meshwork (about 0.15 μ) between spines of the spinose band is detectable though not clear—the difference in resolution here being due to differences in degree of contrast. This is merely a graphic demonstration of the well-known fact that the ability to see objects depends not only on the optical system used but also on the question of what one is trying to see. Separation of two closely spaced spots is more difficult than recognition of a single structure of the same dimensions. The visible-light microscope has a limit of resolution of 0.2 μ but single structures much smaller than this can be seen especially if one of the two dimensions is large and the contrast is high. With the electron microscope such minute structures can be seen clearly and measured accurately.

SUMMARY

1. Electron micrographs of the intima (cuticular lining) of tracheæ of the house mosquito, the honey bee and the American cockroach show structural details extending approximately to 0.015 μ.

2. The thickness of the intertænidial membrane of larger tracheæ of these species is 0.01–0.02 μ after drying; it seems probable that it averages less than 0.02 μ in life. The thickness of the intertænidial membrane of tracheoles after drying seems to be considerably less than 0.01 μ and may be only 0.005 μ.

3. Electron micrographs of tracheæ show no pores in the thin membrane. Since resolution in the best micrographs is at least to 0.004 μ, it is probable that the dry tracheal intima has no pores of this magnitude.

4. Tracheoles (honey bee) less than 0.2 μ in diameter have a helical tænidium 0.02–0.03 μ wide. Thus tracheoles, as well as tracheæ, are shown to possess supporting tænidia. This point can no longer be used to differentiate between tracheæ and tracheoles.

5. Some tracheæ of both adult mosquitoes and cockroaches show tænidia as helices, incomplete rings and complete rings.

6. Some stereoscopic micrographs of tracheæ from shed skins of mosquito larvæ show one spiral inside another. This indicates that the tracheal intima is not necessarily all withdrawn at each moult.

7. Tracheæ of mosquito larvæ have minute spines averaging 0.10 μ in length on the tænidia of the main longitudinal tracheæ. Segmentally arranged, bilaterally symmetrical spinose bands interrupt the tænidia of these main longitudinal trunks just posterior to the origin of the lateral tracheæ.

8. Tracheæ of mosquito larvæ and pupæ differ in that all larval tracheæ observed have uniform intertænidial membranes whereas some pupal tracheæ show reticulate thickenings. Accordingly the tracheal pattern is not necessarily constant throughout the life cycle of the species.

9. The tracheal intima of the honey bee shows complicated thickenings of several types.

10. The tracheal intima of the cockroach shows small swollen knobs.

11. It does not seem likely that these patterns could all originate from foldings of the intima; it is suggested that polymer phenomena may be involved in the development of these thickenings, in the development of tænidia, and in the development of uniform thin membranes.

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PLATE VIII

Figure 1. Electron micrograph of portion of longitudinal tracheal trunk of mosquito larva. 200 kV electrons. Magnification 6,550 ×.

Figure 2. Photomicrograph of same. Magnification 1,450 ×.

Figure 3. Electron micrograph of portion of same. 100 kV electrons. Magnification 14,550 ×.

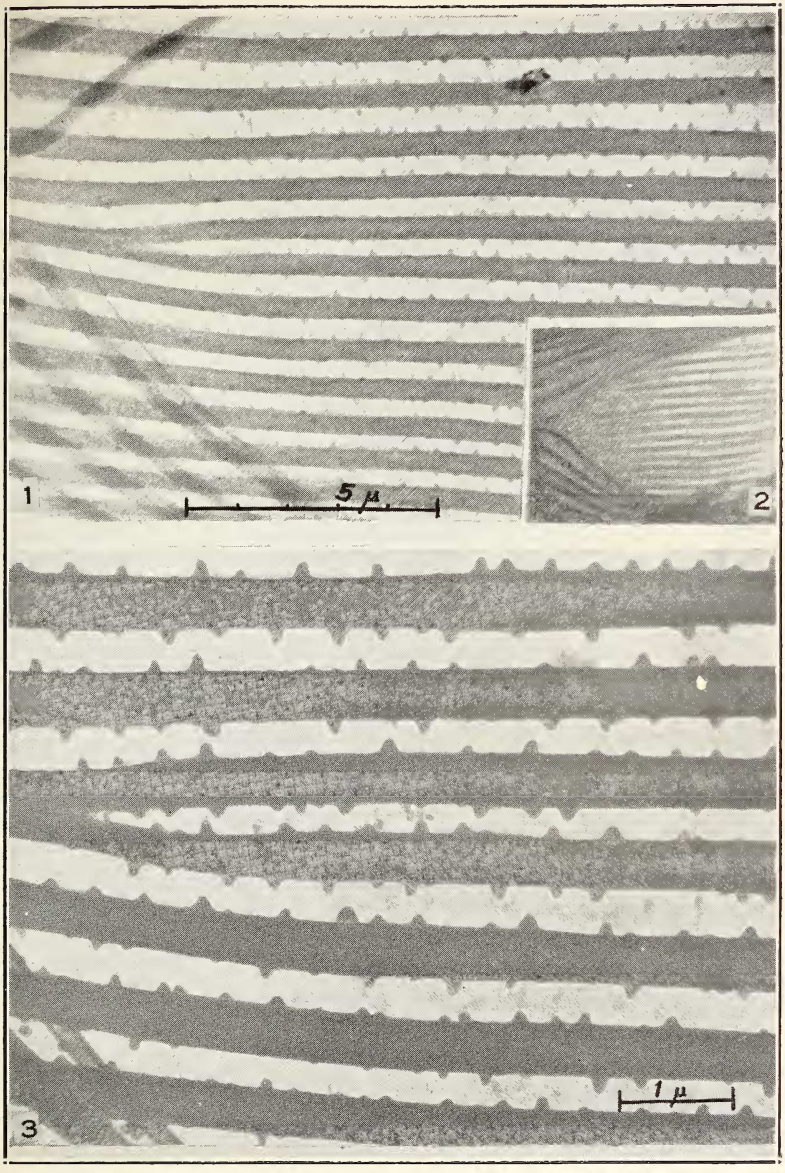

1
5 μ.
2
3
1 μ

PLATE IX

Figure 4. Electron micrograph of portion of spinose band from longitudinal tracheal trunk of mosquito larva. 200 kV electrons. Magnification 7,500 ×.

Figure 5. Micrograph of parts of two of the lateral tracheæ that arise from the longitudinal tracheal trunk of mosquito larva. 100 kV electrons. Magnification 9,500 ×.

Figure 6. Micrograph of another specimen of same. 60 kV electrons. Magnification 9,250 ×.

Figure 7. Micrograph of another specimen of same. This is one of a pair of stereoptic pictures; examination with a stereopticon shows clearly that these have not collapsed. The arrow points to a hole in the collodion membrane where the density of the tracheal membrane can be readily compared with that of the collodion membrane. 60 kV electrons. Magnification 9,250 ×.

Figure 8. Micrograph of portion of trachea from mosquito pupa. (a) and (b) different parts of same micrograph. 60 kV electrons. Magnification 6,500 ×.

Figure 9. Micrograph of portion of trachea from mosquito adult. Central part of picture dirty. Note some of taenidia are rings instead of helices. 60 kV electrons. Magnification 2,720 ×.

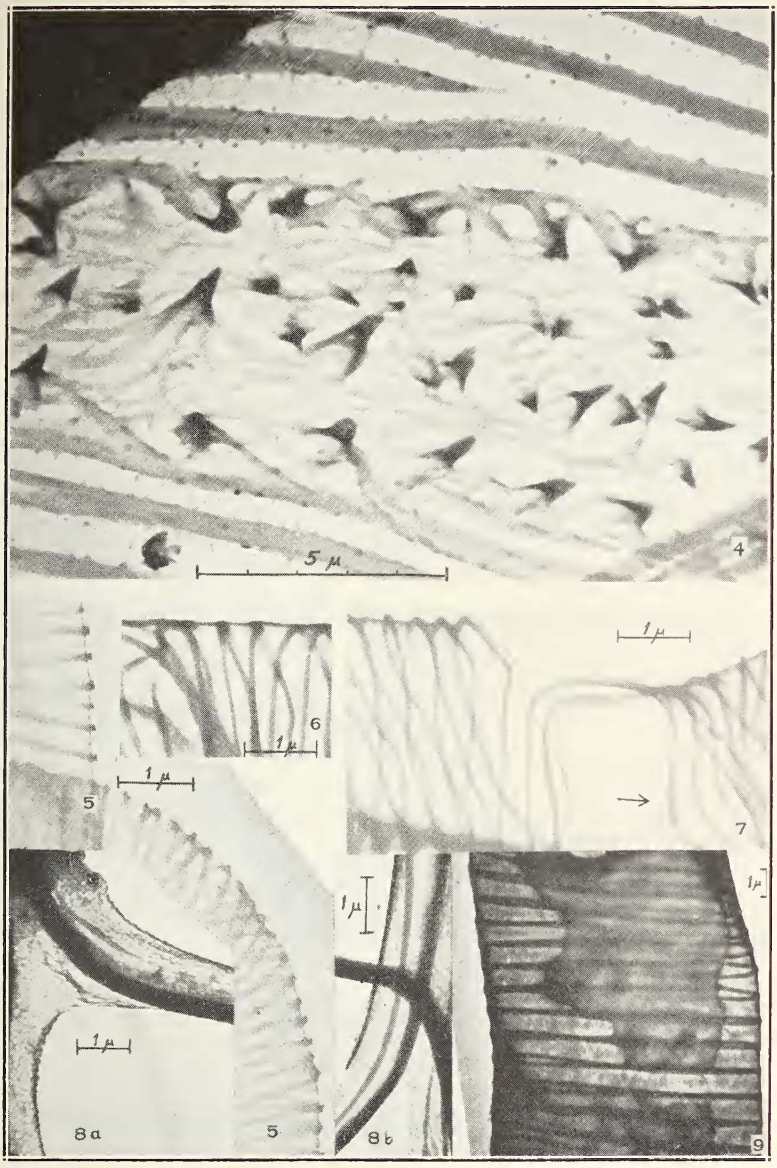

5 μ
4
6
1 μ
1 μ
5
1 μ
7
1 μ
1 μ
8a
5
8b
9

PLATE X

Figure 10. Electron micrograph of portion of trachea of honey bee. 60 kV electrons. Magnification 16,000 ×.

Figure 11. Micrograph of small portion of another trachea of honey bee. 60 kV electrons. Magnification 25,500 ×.

Figure 12. Micrograph of small portion of wall of abdominal air-sac of honey bee. 60 kV electrons. Magnification 11,100 ×.

Figure 13. Micrograph of small portion of a large trachea of cockroach. The dark line on the left side is the edge of a tænidium (tænidia slightly more than 1 μ broad in this trachea). 60 kV electrons. Magnification 12,000 ×.

Figure 14. Micrograph of a small trachea of cockroach. Note tænidia as helices, incomplete rings and complete rings. 60 kV electrons. Magnification 12,000 ×.

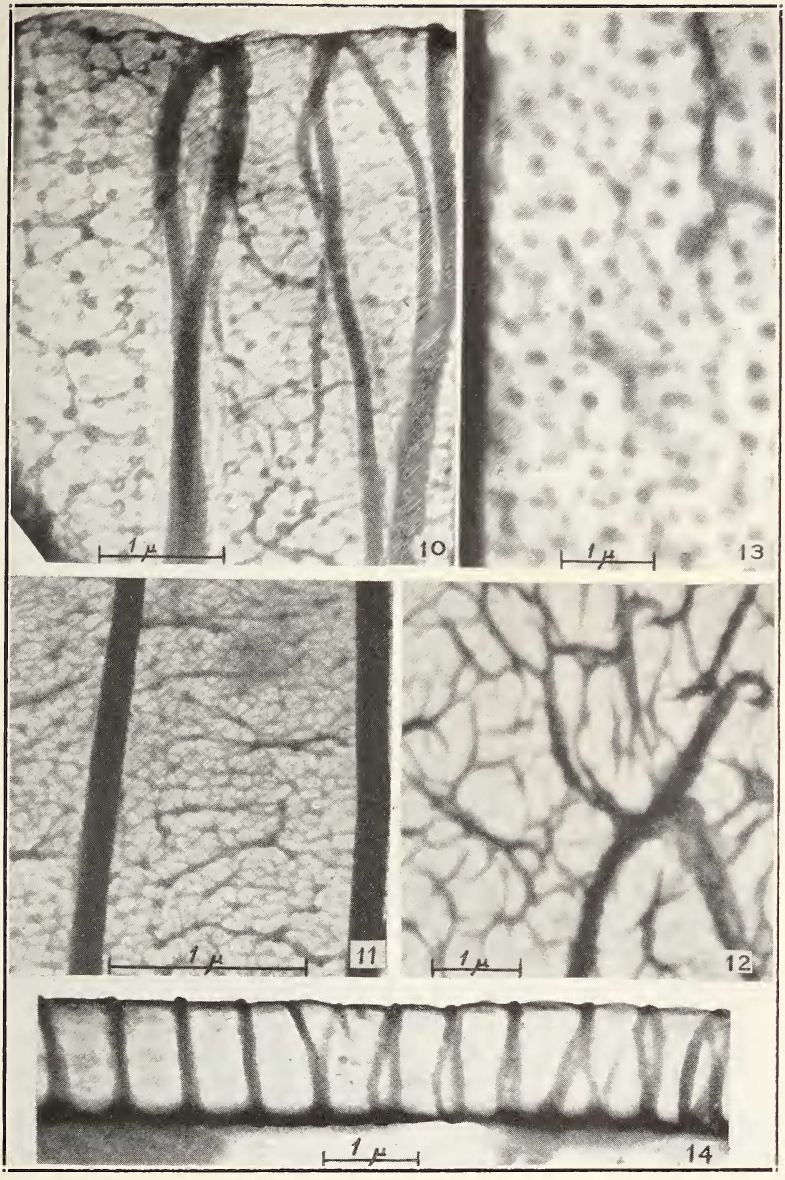
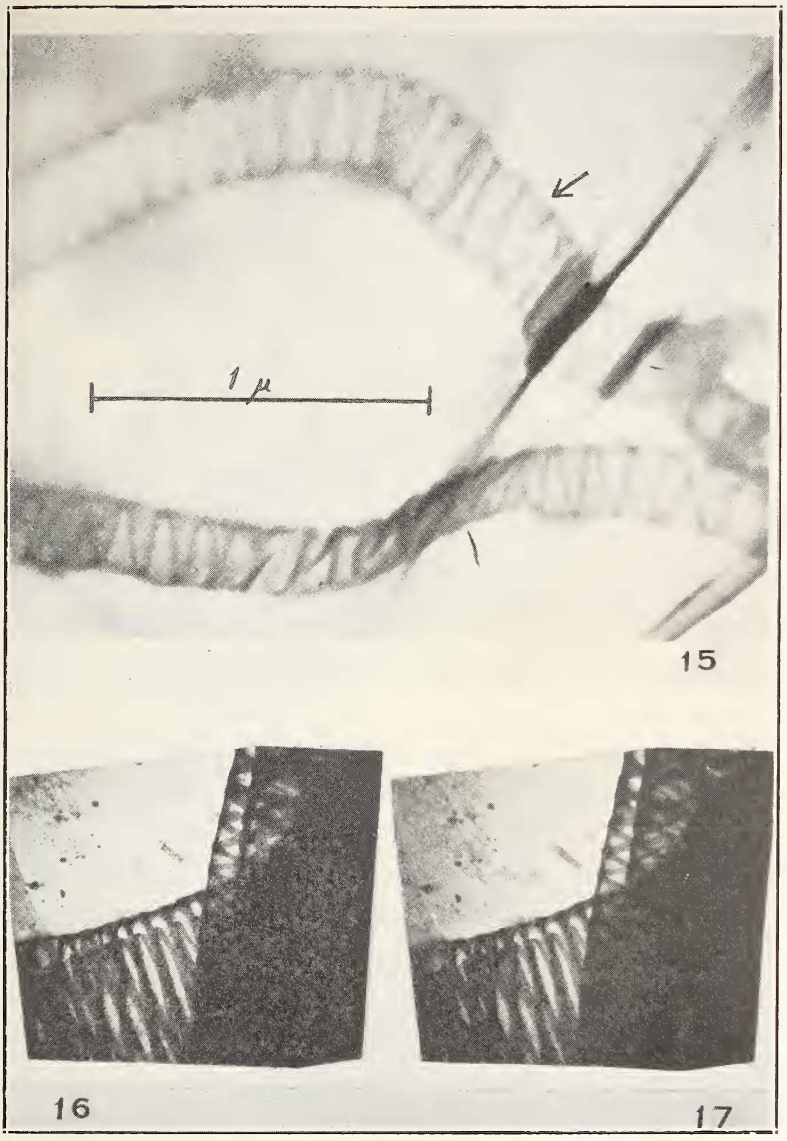

1 μ
10
1 μ
13
1 μ
11
1 μ
12
1 μ
14

PLATE XI

Figure 15. Electron micrograph of tracheole of honey bee. This tracheole collapsed; its calculated diameter is less than 0.2 μ. The arrow points to double silhouette line where the thickness of the tracheole wall can be measured. 60 kV electrons. Magnification 44,400 ×.

Figure 16–17. Stereo electron micrographs of tracheæ from shed skin of third instar mosquito larva. These may be viewed directly with a stereopticon to show that one tube is inside the other. 60 kV electrons. Magnification 2,720 ×.


1 μ
15
16
17